

I

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/24785 A2

(51) International Patent Classification⁷: **A61K 31/00, 31/47, 31/4709, 31/497, 31/473, 31/4184, 31/122, 31/196, C07D 215/52, A61P 31/14, A61K 38/21, 31/711, 31/343, 31/437 // (A61K 38/21, 31:00) (A61K 31/711, 31:00) (A61K 31/437, 31:00) (A61K 31/343, 31:00)**

(74) Agent: WILSON, Mary, J.; Nixon & Vanderhye PC, Suite 800, 1100 North Glebe Road, Arlington, VA 22201-4714 (US).

(21) International Application Number: PCT/US00/26797

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(22) International Filing Date:

29 September 2000 (29.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(30) Priority Data:
60/157,017 1 October 1999 (01.10.1999) US

Published:

— Without international search report and to be republished upon receipt of that report.

(71) Applicant (for all designated States except US): INSTITUTE OF MOLECULAR AND CELL BIOLOGY [SG/SG]; 30 Medical Drive, Singapore 117609 (SG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors; and

(75) Inventors/Applicants (for US only): TAN, Yin, Hwee [CA/SG]; 30 Medical Drive, Singapore 117609 (SG). DRISCOLL, John, Stanford [US/US]; 4 Infield Court South, Rockville, MD 20854 (US). MUI MUI, Sim [SG/SG]; 30 Medical Drive, Singapore 117609 (SG).



WO 01/24785 A2

(54) Title: COMPOUNDS FOR THE TREATMENT OF VIRAL-MEDIATED DISEASES

(57) Abstract: Flavivirus, rhabdovirus and paramyxovirus infections may be treated by administering an inhibitor of the enzyme dihydroorotate dehydrogenase such as 6-fluoro-2-(2'-fluoro-1,1'-biphenyl-4-yl)-3-methyl-4-quinolinesearcarboxylic acid sodium salt (Brequinar). A synergistic effect can be obtained if an interferon such as interferon α 2, interferon α 8 or interferon β , or an inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase, is also administered.

-1-

COMPOUNDS FOR THE TREATMENT OF
VIRAL-MEDIATED DISEASES

Field of the Invention

5 This invention relates to the treatment of infections caused by viruses of the Flaviviridae, Rhabdoviridae and Paramyxoviridae families.

Background to the Invention

At present there is no effective treatment for chronic hepatitis C. So far, 10 human interferons have been used to treat patients with chronic hepatitis C. The efficacy of this treatment is about 20%. Recently, Ribavirin has been used in combination with interferon to treat patients with chronic hepatitis C. The combined treatment efficacy is up to about 40%. However, the dosage of Ribavirin required is high and there is accompanying red blood cell toxicity.

15 The search for effective drug(s) for hepatitis C is under the scrutiny of investigators worldwide. Most if not all the enzymes of hepatitis C and related viruses of the Flaviviridae family have been used as targets in the search for an antiviral drugs against hepatitis C. A major problem with the study of hepatitis C is a lack of *in vitro* cell-based and animal model systems. To date, there is no good 20 replicative cell system to assay for activity against hepatitis C virus. The only animal model for hepatitis C is the chimpanzee. However, chronic hepatitis C infection is difficult to establish in chimpanzees. This fact further complicates the use of chimpanzees as an animal model system.

25 **Summary of the Invention**

"Surrogate" virus/host cell systems were used to search for antiviral 30 compounds which can be used as antiviral drugs to treat patients with acute and chronic hepatitis C in particular, and flavivirus, rhabdovirus and paramyxovirus infections in general. Several families of compounds which are structural and biological analogues were selected for testing for their antiviral activities in human and monkey cells against three viruses of the Flaviviridae family (yellow fever,

-2-

kunjin and dengue viruses), a virus of the Rhabdoviridae family (vesicular stomatitis virus; VSV) and a virus of the Paramyxoviridae family (respiratory syncytial virus; RSV). These are all RNA viruses that infect human and non-human primate cells.

It was found that the 6-fluoro-2-(2'-fluoro-1,1'-biphenyl-4-yl)-3-methyl-4'-quinolinecarboxylic acid sodium salt (Brequinar) and other inhibitors of the enzyme dihydroorotate dehydrogenase exhibited potent activity against the viruses tested. Ribavirin was also tested. The dihydroorotate dehydrogenase inhibitors were always more potent than, and indeed act synergistically in combination with, an interferon. An inhibitor of dihydroorotate dehydrogenase can also be administered in combination therapy with an inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase, optionally in further combination with an interferon, to provide a synergistic antiviral effect.

Accordingly, the present invention provides a method of treating a host infected with a virus of the Flaviviridae, Rhabdoviridae or Paramyxoviridae family, which method comprises the step of administering to the host an inhibitor of dihydroorotate dehydrogenase.

The invention additionally provides:

- novel compounds which are structural analogues of Brequinar and their preparation;
- use of an inhibitor of dihydroorotate dehydrogenase in the manufacture of a medicament for use in the treatment of an infection attributable to a virus of the Flaviviridae, Rhabdoviridae, or Paramyxoviridae family;
- 25 - an anti-flavivirus agent, anti-rhabdovirus or anti-paramyxovirus agent comprising an inhibitor of dihydroorotate dehydrogenase;
- products containing an inhibitor of dihydroorotate dehydrogenase and an interferon as a combined preparation for simultaneous, separate or sequential use in treating an infection attributable to a virus of the
- 30 - Flaviviridae, Rhabdoviridae or Paramyxoviridae family;
- products containing an inhibitor of dihydroorotate dehydrogenase and an

-3-

inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase as a combined preparation for simultaneous, separate or sequential use in treating an infection

5 attributable to a virus of the Flaviviridae, Rhabdoviridae or Paramyxoviridae family; and

- A method for identifying an anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent, which method comprises testing a test compound for its ability to inhibit dihydroorotate dehydrogenase.

10

Detailed Description of the Invention

Inhibitors of dihydroorotate dehydrogenase

15 Dihydroorotate dehydrogenase (DHO-DH, DHOD, EC 1.3.3.1) is the fourth enzyme in the *de novo* pyrimidine biosynthetic pathway. Any inhibitor of this enzyme is used in the present invention.

A technique for identifying inhibitors of dihydroorotate dehydrogenase using a computer algorithm is described in Biochemical and Biophysical Research Communications 223, 654-659 (1996) and in Biochemical Pharmacology vol 49, No. 20 7, pp 947-954 (1995). The technique involves the correlation of the biological activity of a given compound with the biological activity of known DHOD inhibitors such as dichloroallyl lawsone and Brequinar. The COMPARE computer algorithm may be used for the correlation (see J. Natl. Cancer Inst. 81, 1088-1092, 1989).

An *in vitro* assay for inhibitors of mouse liver dihydroorotate dehydrogenase is described in J. Biol. Chem. 270, No. 38, pages 22467-22472 (1995). Mouse liver dihydroorotate dehydrogenase may be prepared as described in Biochem. J. (1998), 336, 299-303 (1998).

Inhibitors of dihydroorotate dehydrogenase are described, for example, by Douglas G. Batt in Exp. Opin. Ther. Patents (1999) 9 (1), 41-54, the contents of 30 which are incorporated herein by reference.

The fact that inhibitors of dihydroorotate dehydrogenase have been found to

-4-

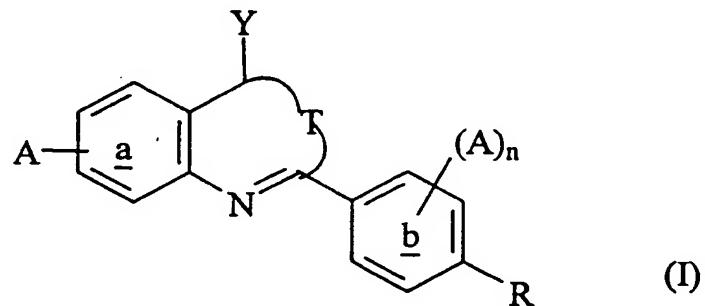
have activity against certain classes of RNA virus offers a means of screening for new antiviral agents. Accordingly the invention provides a method of identifying an anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent, which method comprises:

- 5 (a) providing a test compound;
- (b) determining whether the test compound has activity as an inhibitor of dihydroorotate dehydrogenase; and
- (c) selecting the test compound as an anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent if it is shown to have activity in step (b).

10 The candidate test compounds may be tested for dihydroorotate dehydrogenase inhibitory activity by any known technique, such as those mentioned above.

Four classes of dihydroorotate dehydrogenase inhibitors are preferred for use in the present invention. These are:

15 compounds of the formula (I):



wherein:

each A is independently selected from the group consisting of hydrogen, halogen, perhaloalkoxy, amino C₁-C₈ alkyl, NO₂, CN, SO₂CH₃, C₁-C₈ alkyl, C₁-C₈ alkoxy, C₃-C₇ cycloalkyl, C₃-C₇ cycloalkenyl, aryl, aryloxy, C₁-C₆ perhaloalkyl and Y; or

20 two adjacent groups A on ring b form, together with the phenyl ring to which they are attached, a naphthalene ring system;

R is cyclohexyl, phenoxy or benzoxy, or a phenyl ring which is unsubstituted or

-5-

substituted by a group A as defined above; or

R and an adjacent group A on ring b form, together with the phenyl ring to which they are attached, a naphthalene or phenanthrene ring system;

Y is selected from the group consisting of COOM, CONHR', SO₃M and hydrogen;

5 M is selected from the group consisting of H, Li, Na, K and 0.5 Ca;

R' is C₁-C₁₀ alkyl;

n is 1 or 2; and

T is =N- or =C(Z)- wherein either:

(i) Z is selected from the group consisting of hydrogen, NH₂, OH, C₁-C₈

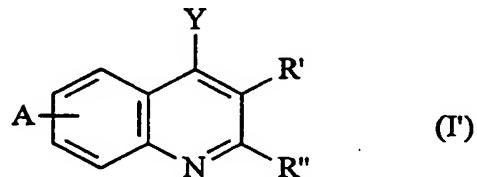
10 alkyl, C₃-C₇ cycloalkyl, aryl and C₁-C₆ perhaloalkyl, or

(ii) Z is a bridging moiety selected from the group consisting of -V-W-

(wherein V is CH₂ or S and W is CH₂, O, S or NH) and -(CH₂)₂-C(=Z)- wherein Z is O or H₂, the said bridging moiety being attached to the ortho position of ring b of the adjacent biphenyl group, thereby completing a ring;

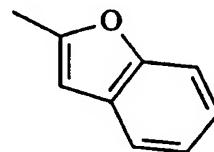
15

- compounds of formula (I'):



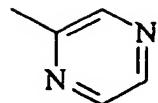
wherein A and Y are as defined above for formula (I);

R' is hydrogen and R'' is a thiophene ring or a group of formula (i') or (ii'):



(i')

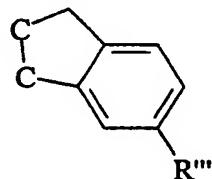
-6-



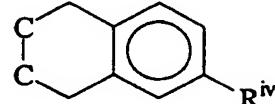
(ii')

or R' and R" form, together with the carbon atoms (denoted "C") to which they are attached, a ring system of formula (iii') or (iv'):

5



(iii')

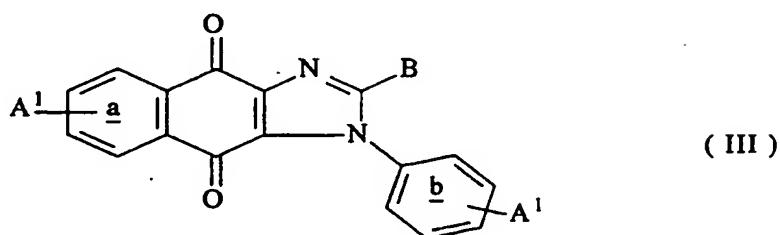


(iv')

10

wherein R''' is H or halogen and R^{iv} is H or C₁ - C₆ alkoxy;

15 - compounds of formula (III):



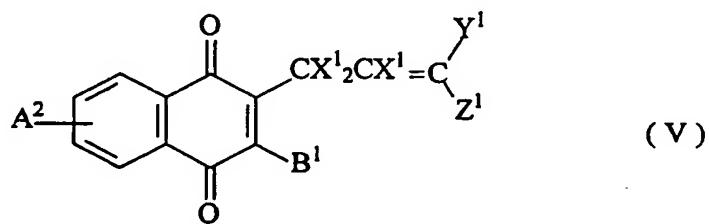
wherein:

each A¹ is independently selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₈ alkoxy, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₇ cycloalkyl, halogen,

-7-

unsubstituted aryl, X-substituted aryl, NO₂, CN, COOR, CONHR and NHR;
 X is selected from the group consisting of halogen, NO₂, C₁-C₈ alkyl, aryl, fused aryl and COOR;
 R is selected from the group consisting of hydrogen and C₁-C₈ alkyl; and
 5 B is selected from the group consisting of C₁-C₈ alkyl, H, CF₃ and aryl which is unsubstituted or substituted by halogen, C₁-C₈ alkoxy, C₁-C₈ alkyl, NO₂, aryl or fused aryl;

- compounds of the formula (V):

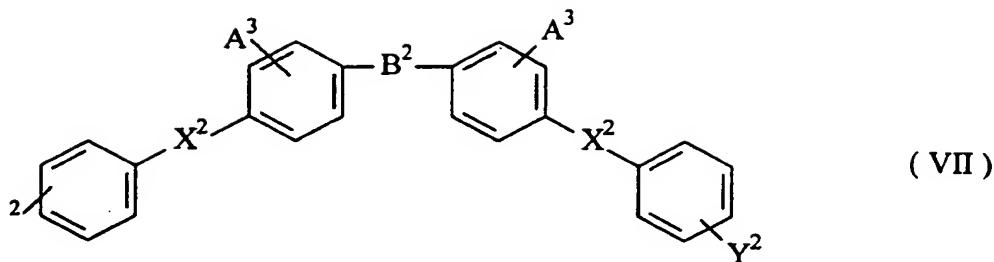


10 wherein:

A² is selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₇ cycloalkyl, halogen, unsubstituted aryl, halogen-substituted aryl, fused aryl, NO₂, CN, NHR¹ and N(R¹)₂;
 R¹ is selected from the group consisting of hydrogen, C₁-C₈ alkyl and OH;
 15 X¹ is hydrogen or halogen; and
 B¹, Y¹ and Z¹ are each independently selected from hydrogen, OH, C₁-C₈ alkyl, halogen, CN, NO₂ and CF₃; and

- compounds of the formula (VII):

-8-



wherein:

each A³ is independently selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₁₀ alkoxy, halogen and N(R²)₂;

5 B² is a direct bond, -CH=CH- or -C=C-;

X² is selected from the group consisting of O, S and NR²;

R² is selected from the group consisting of hydrogen, C₁-C₄ alkyl and aryl;

Y² is selected from the group consisting of COOM¹ and SO₃M¹; and

M¹ is selected from the group consisting of H, Li, Na, K and 0.5 Ca.

10 A halogen atom may be fluoro, chloro, bromo or iodo. A C₁-C₈ alkyl group is suitably a C₁-C₄ alkyl group. A C₁-C₄ alkyl group is typically methyl, ethyl, n-propyl, isopropyl or butyl. A C₃-C₇ cycloalkenyl group is typically cyclohexenyl. A C₃-C₇ cycloalkyl group is typically a cyclopentyl or cyclohexyl group. A C₁-C₆ perhaloalkyl group may be a C₁-C₄ perhaloalkyl group. The halo atom may be chloro or fluoro. A particularly suitable perhaloalkyl group is trifluoromethyl.

15 An aryl group is typically a phenyl. A C₂-C₈ alkenyl group is suitably a C₂-C₄ alkenyl group. A C₂-C₈ alkynyl group is suitably a C₂-C₄ alkynyl group. Fused aryl is generally naphthyl. A C₁-C₁₀ alkoxy group is preferably a C₁-C₆ alkoxy group, for example a C₁-C₄ alkoxy group such as methoxy or ethoxy.

20 Perhaloalkoxy is, for example, OCF₃, OCCL₃ or OCBr₃. Preferably it is OCF₃. Aryloxy is, for example, phenoxy or benzyloxy.

The compounds of formula (I) are known and may be prepared by published methods or by submitting compounds produced by the published methods to routine synthetic modifications and interconversion which are well known in organic

25 chemistry. Literature references for many of the published methods are quoted in the paper by Douglas G. Batt cited above. For instance, compounds of formula (I) in

-9-

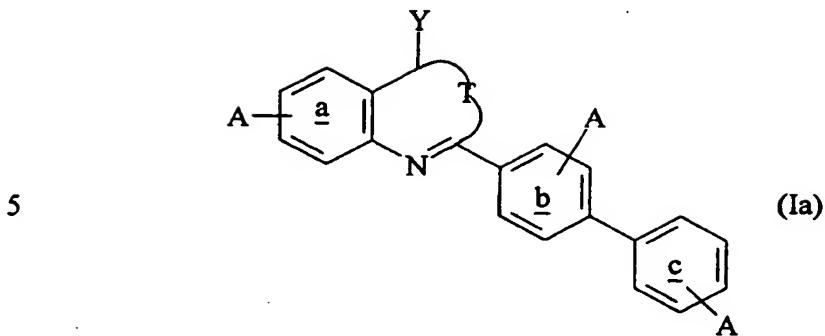
which T is $=C(Z)-$ may be prepared as described in US-A-4680299. Compounds of formula (I) in which T is $=N-$ may be prepared as described in US-A-4639454. Compounds of formula (I) in which Z is a bridging moiety as defined above may be prepared as described in US-A-4918077, US-A-5002954, 5 WO9506640, US-A-5371225, EP-A-721942, JP10231289, Organ Biol. (1997) 4(2): 43-48 and 49-57, JP-6306079-A2 and 216th ACS Meeting, Boston USA (1998) ORGN 132. Compounds of formula (I') may be prepared using the same synthetic strategy as that described in US-A-4680299.

Preferred compounds of formula (I) are those in which:

- 10 each A is independently selected from the group consisting of hydrogen, halogen (preferably F) amino C_1 - C_8 alkyl (preferably $NH_2(CH_2)-$), C_1 - C_8 alkyl (preferably CH_3) and C_1 - C_6 perhaloalkyl (preferably CF_3);
Y is selected from the group consisting of $COOM$ and $CONHR'$ wherein M is as defined above and R' is C_5 - C_{10} alkyl, preferably octyl;
- 15 T is $=C(Z)-$ wherein
 - (i) Z is selected from the group consisting of hydrogen, C_1 - C_8 alkyl (preferably CH_3), NH_2 and OH , or
 - (ii) Z is a bridging moiety as defined above selected from the group consisting of $-(CH_2)-$, $-(CH_2)_3-$, $-SCH_2-$, $-CH_2O$, $-CH_2S-$, $-CH_2NH-$ and $-(CH_2)-C(=O)-$.
- 20 In formula (I) the substituent A in ring a is preferably OCF_3 , halogen (most preferably F) or $NH_2(CH_2)_2-$, preferably bonded at position 6 of the quinoline ring system. The substituent A in ring b is preferably hydrogen.

Examples of compounds of formula (I) include those of formula (Ia):

-10-



10

wherein:

each A is independently selected from the group consisting of hydrogen, halogen, amino C_1 - C_8 alkyl, NO_2 , CN , SO_2CH_3 , C_1 - C_8 alkyl, C_3 - C_7 cycloalkyl, aryl, C_1 - C_6 perhaloalkyl and Y;

15 Y is selected from the group consisting of $COOM$, $CONHR'$ SO_3M and hydrogen;

M is selected from the group consisting of H, Li, Na, K and 0.5 Ca;

R' is C_1 - C_{10} alkyl; and

T is $=N-$ or $=C(Z)-$ wherein either:

20 (i) Z is selected from the group consisting of hydrogen, NH_2 , OH , C_1 - C_8 alkyl, C_3 - C_7 cycloalkyl, aryl and C_1 - C_6 perhaloalkyl, or

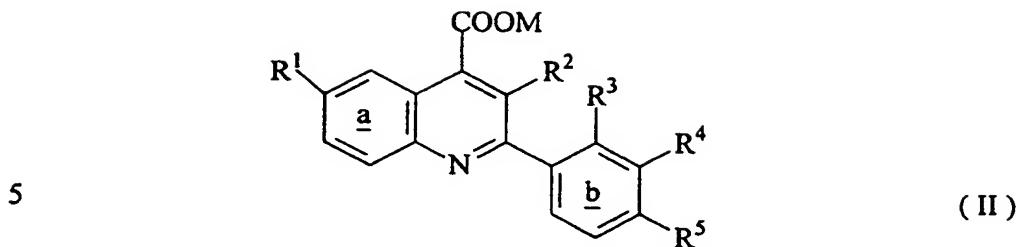
(ii) Z is a bridging moiety selected from the group consisting of -V-W- (wherein V is CH_2 or S and W is CH_2 , O, S or NH) and $-(CH_2)_2-C(=Z)-$ wherein Z is O or H_2 , the said bridging moiety being attached to the ortho position of ring b of the

25 adjacent biphenyl group, thereby completing a ring.

The substituent A in ring c of formula (Ia) is preferably hydrogen or ortho-halogen (most preferably ortho-F).

Amongst preferred compounds of formula (I) are those of formula (II):

-11-



10 wherein

R¹ is H, a halogen or OCF₃;

M is as defined above;

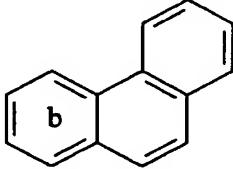
R² is H or C₁-C₆ alkyl;R³ is H or OR⁶ wherein R⁶ is H or C₁-C₆ alkyl;15 R⁴ is H or C₁-C₆ alkyl; or R³ and R⁴ form, together with phenyl ring b to which they are attached, a naphthalene ring; andR⁵ is cyclohexyl, phenoxy or benzoxy, or a phenyl ring which is unsubstituted or substituted by halogen; or R⁴ and R⁵ form, together with phenyl ring b to which they are attached, a phenanthrene ring.

20

Substitution patterns of specific compounds of formula (II) are as follows:

R ¹	M	R ²	R ³	R ⁴	R ⁵	Compound
F	H	CH ₃	H	H	2-fluoro-phenyl	Brequinar
F	H	H	H	H	phenyl	I3K5
F	H	CH ₃	H	H	phenyl	I3K55
F	H	H	H	H	cyclohexyl	I3K46
F	H	H	H	H	phenoxy	I3K52
F	H	H	OH	CH ₃	benzoxy	I3K53
H	H	H	H	H	phenoxy	I1K52

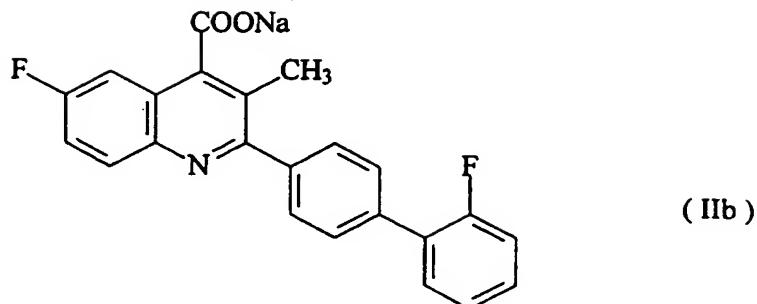
-12-

	H	H	H	H	H	cyclohexyl	I1K46
	H	H	H	OH	CH ₃	benzoxy	I1K53
	H	H	CH ₃	H	H	phenyl	I1K55
	OCF ₃	H	H	H	H	phenyl	I2K5
5	F	H	H	H	form with ring b a phenanthrene ring of formula:		I3K4
							
	F	H	H	form with ring b a naphthalene ring		F	I3K36
	OCF ₃	H	H	H	H	cyclohexyl	I2K46
	OCF ₃	H	CH ₃	H	H	phenyl	I2K55
	OCF ₃	H	H	OCH ₃	CH ₃	benzoxy	I2K51
10	OCF ₃	H	H	H	H	phenoxy	I2K52
	F	H	H	OCH ₃	CH ₃	benzoxy	I3K51
	F	H	H	form with ring b a naphthalene ring		H	I3K6

Especially preferred is the sodium salt of Brequinar, which has the formula

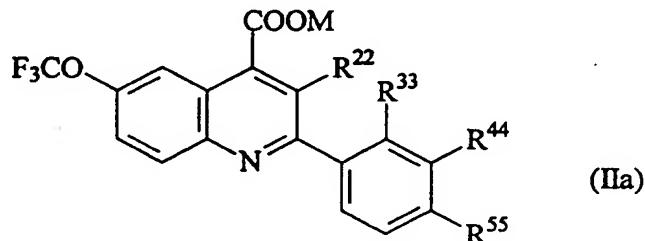
15 (IIb):

-13-



Brequinar can be prepared as described in US-A-4680299 (Example 28).

Some of the compounds of formula (II) are novel. Accordingly, the invention further provides a compound of formula (IIa)



wherein

5 M is selected from the group consisting of H, Li, Na, K and 0.5 Ca;

R²² is H or C₁-C₆ alkyl;

R³³ is H or OR⁶ wherein R⁶ is H or C₁-C₆ alkyl;

R⁴⁴ is H or C₁-C₆ alkyl; and

R⁵⁵ is phenyl, cyclohexyl, phenoxy or benzoxy.

10 Preferred examples of the compounds of formula (IIa) are:

2-(4-biphenylyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K5);

2-(4-biphenylyl)-3-methyl-6-trifluoromethoxy-quinoline-4-carboxylic acid

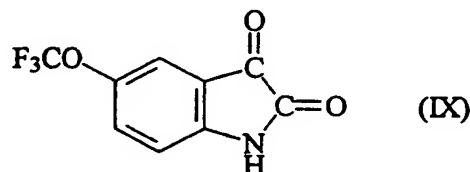
-14-

(compound I2K55);
 2-(4-cyclohexylphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K46);
 2-(4-benzyloxy-2-methoxy-3-methyl-phenyl)-6-trifluoromethoxy-quinoline-4-
 5 carboxylic acid (compound I2K51); and
 2-(4-phenoxyphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K52).

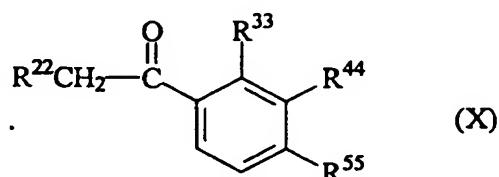
The compounds of formula (II), including the novel compounds of formula (IIa), may be prepared by the synthetic route described in US-A-4680299 mentioned
 10 above. This involves the Pfitzinger reaction, whereby an appropriately substituted
 isatin is condensed with an appropriately substituted ketone (J. Org. Chem. 18, 1209,
 1953).

Accordingly, the invention further provides a process for producing a
 compound of formula (IIa) as defined above, which process comprises
 15 a) condensing the trifluoromethoxy-substituted isatin compound of the
 following formula (IX):

20



25 with a ketone of formula (X):



wherein R²², R³³, R⁴⁴ and R⁵⁵ are as defined above for formula (IIa), in the presence

-15-

of a base; and

(b) if desired, converting a resulting compound of formula (IIa) in which M is H into a pharmaceutically acceptable salt thereof wherein M is Li, Na, K or 0.5 Ca.

5 The base used in step (a) may be an inorganic base or an organic base. Preferred bases include potassium hydroxide and sodium hydroxide. The reaction is generally conducted in a solvent. The solvent is preferably ethanol.

Preferred compounds of formula (I') have the following substitution patterns:

	A	Y	R'	R''	Compound
10	6-F	COOH	H	formula (i')	I3K44
	6-OCF ₃	COOH		formula (iv') wherein R ^{iv} is methoxy	I2K42
	6-OCF ₃	COOH		formula (iii') wherein R''' is F	I2K43
	6-F	COOH		formula (iv') wherein R ^{iv} is methoxy	I3K42
15	6-OCF ₃	COOH	CH ₃	thiophen-2-yl	I2K20
	6-F	COOH		formula (iii') wherein R''' is F	I3K43
	H	COOH	H	formula (i')	I1K44

Preferred compounds of formula (III) are those in which:

20 each A' is independently selected from the group consisting of hydrogen, halogen, C₁-C₈ alkyl and C₁-C₈ alkoxy; and
 B is selected from the group consisting of hydrogen, C₁-C₈ alkyl, phenyl and benzyl, the said phenyl and benzyl being unsubstituted or substituted by halogen (preferably Cl or Br), C₁-C₈ alkoxy (preferably C₁-C₄ alkoxy such as OCH₃) or C₁-C₈ alkyl (preferably C₁-C₄ alkyl such as CH₃).

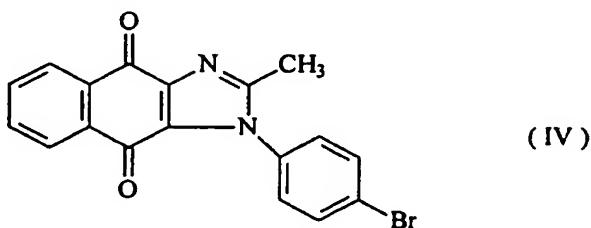
When B is substituted phenyl or benzyl as defined above, the substituent is preferably at position 4 of the phenyl ring. The group A' in ring a is preferably H. The group A' in ring b is preferably H, or it is para-C₁-C₈ alkoxy or para-halogen

-16-

(preferably Cl or Br).

Especially preferred is 1-(p-bromophenyl)-2-methyl-1H-naphth[2,3-d]imidazole-4,9-dione (BNID) which has the formula (IV):

5



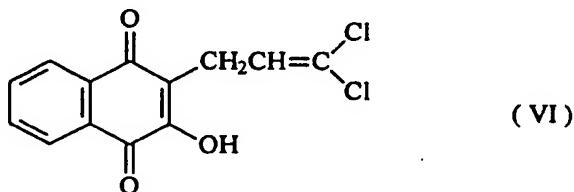
10 The compounds of formula (III) may be prepared as described in J. Med. Chem. 1996, 39, 1447-1451, or by submitting compounds produced as described in this document to routine synthetic modifications and interconversion which are well known in organic chemistry. BNID can be prepared as described in J. Med. Chem. 1964, 7, 362-364.

15 Preferred compounds of formula (V) are those in which A² is selected from the group consisting of hydrogen, C₁-C₈ alkyl (preferably C₁-C₄ alkyl such as CH₃) and halogen; B is hydrogen or OH; X¹ is hydrogen; and

20 Y¹ and Z¹ are each independently selected from the group consisting of hydrogen, halogen (preferably Cl) and C₁-C₈ alkyl (preferably C₁-C₄ alkyl such as CH₃).

Especially preferred is dichloroallyl lawsone which has the formula (VI):

25



30 The compounds of formula (V) may be prepared as described in US-A-3655699 or WO9106863, or by submitting compounds produced as described in these documents to routine synthetic modifications and interconversions which are well known in organic chemistry. Dichloroallyl lawsone can be prepared as

-17-

described in US-A-3655699.

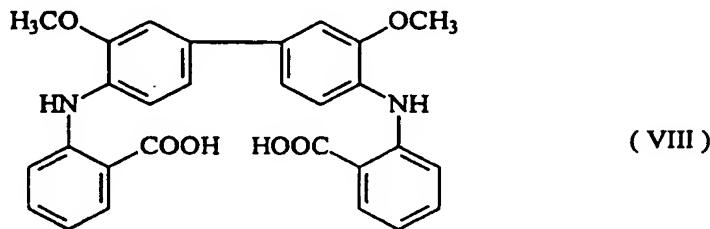
Preferred compounds of formula (VII) are those in which each A³ is hydrogen or C₁-C₁₀ alkoxy (preferably C₁-C₆ alkoxy such as methoxy); B² is a direct bond;

5 X² is NR² wherein R² is hydrogen or C₁-C₄ alkyl; and Y² is COOH.

Preferably each A³ in formula (VII) is positioned meta to the linking group B², and each Y² is positioned ortho to the linking group X².

Especially preferred is 2,2'-[3,3'-dimethoxy[1,1'-biphenyl]-4,4'-diyl]diimino]bis-benzoic acid (redoxal) which has the formula (VIII):

15



Redoxal is described in Zhur. Anal. Khim. 15, pp 671-675, 1960 and Chemical Abstracts 1961 vol. 5, 18447b. Other compounds of formula (VII) may be prepared

20 by routine synthetic modifications and interconversion of compounds prepared in this reference.

Interferons

The interferon for use in the present invention may be an interferon α , such as

25 interferon $\alpha 2$ or $\alpha 8$, or interferon β . The term "interferon" includes fragments which have interferon activity and mutant forms of an interferon which retain interferon activity. For example, the sequence of an interferon α or β may have been modified to enhance activity or stability as reported in US-A-5582824, US-A-5593667 or US-A-5594107.

30 The interferon may have been purified from natural sources or may be a recombinant interferon. The species of interferon is generally the same as the host

-18-

species to which the interferon is administered. The invention is particularly applicable to the treatment of flavivirus infections in humans. Preferably therefore a human interferon such as human interferon α 2 or α 8 or human interferon β is used.

The interferon α 8, particularly the human interferon α 8, typically has a 5 specific activity of more than 0.3×10^9 , generally from 0.3×10^9 to 3×10^9 and preferably from 0.5×10^9 to 3×10^9 , IU per mg protein. The human interferon β typically has a specific activity of from 4×10^8 to 8×10^8 , preferably from 4.8×10^8 to 6.4×10^8 , IU per mg protein. Interferon α and interferon β specific activities are determined according to reference standards MRC 69/19 and Gb-23-902-531 10 respectively. Specific activity is determined according to a modification of the method of Armstrong, Applied Microbiology 21, 723, 1971, in which 0.2 μ g/ml of actinomycin D is included in the viral challenge and the viral induced cytopathic effect is read directly.

The interferon such as the interferon α 2 or α 8 or the interferon β is preferably 15 obtainable by the methodology of WO 96/30531. The interferon is thus obtainable by a process comprising culturing mammalian cells transfected with a nucleic acid vector comprising:

- (i) a coding sequence which encodes the interferon and which is operably linked to a promoter capable of directing expression of the coding 20 sequence in mammalian cells in the presence of a heavy metal ion;
- (ii) a first selectable marker sequence which comprises a metallothionein gene and which is operably linked to a promoter capable of directing expression of the metallothionein gene in the cells in the presence of a heavy metal ion; and
- 25 (iii) a second selectable marker sequence which comprises a *neo* gene and which is operably linked to a promoter capable of directing expression of the *neo* gene in the cells;

unless conditions that allow expression of the coding sequence; and recovering the interferon thus produced.

30 The transfected mammalian cells may be cells of a human or animal cell line. They may be BHK, COS, Vero, human fibroblastoid such as C10, HeLa, or human

-19-

lymphoblastoid cells or cells of a human tumour cell line. Preferably, however, the cells are CHO cells, particularly wild-type CHO cells.

Desirably, transfected cells will have all or part of such a vector integrated into their genomes. Such cells are preferred because they give stable expression of 5 the coding sequence contained in the vector. Preferably, one or more copies of the entire vector will be integrated, with cells having multiple integrated copies of the vector, for example from 20 to 100 copies or more, being particularly preferred because these cells give a high stable level of expression of the coding sequence contained in the vector.

10 However, cells having less than complete sections of the vectors integrated into their genomes can be employed if they are functionally equivalent to cells having the entire vector integrated into their genomes, in the sense that the integrated sections of the vector enable the cell to express the coding sequence and to be selected for by the use of heavy metals. Thus, cells exhibiting partial integration of a 15 vector may be employed if the integrated element or elements include the coding sequence operably linked to its associated promoter and the metallothionein marker sequence operably linked to its associated promoter.

Any promoter capable of enhancing expression in a mammalian cell in the presence of a heavy metal ion such as Cd²⁺, Cu²⁺ and Zn²⁺ may be operably linked to 20 the interferon coding sequence. A suitable promoter is a metallothionein gene promoter. The mouse metallothionein gene I (mMT1) promoter is preferred.

Suitable promoter/enhancer combinations for the coding sequence include the mMT1 promoter flanked upstream with a mouse sarcoma virus (MSV) enhancer (MSV-mMT1) and a Rous sarcoma virus (RSV) enhancer upstream of a mouse 25 mammary tumour virus (MMTV) promoter. MSV-mMT1 is preferred.

As far as the first selectable marker sequence is concerned, any promoter capable of enhancing expression in a mammalian cell in the presence of a heavy metal ion such as Cd²⁺, Cu²⁺ and Zn²⁺ may be operably linked to the metallothionein gene such as a human metallothionein gene. Preferably, the marker sequence gene is 30 a human metallothionein gene, such as the human metallothionein gene IIA, which has its own promoter.

-20-

The second selectable marker sequence is a *neo* gene. More than one type of this gene exists in nature: any specific *neo* gene can be used in a vector of the invention. One preferred *neo* gene is the *E.coli* *neo* gene.

The promoter for the *neo* gene is capable of directing expression of the gene 5 in a mammalian cell. Suitable promoters are the cytomegalovirus (CMV) early promoter, the SV40 promoter, the mouse mammary tumour virus promoter, the human elongation factor 1 α -P promoter (EF-1 α -P), the SR α promoter and a metallothionein gene promoter such as mMT1. The promoter may also be capable of expressing the *neo* gene in bacteria such as *E.coli* in which a vector may be 10 constructed.

The interferon coding sequence (i) and the marker sequences (ii) and (iii) are thus each operably linked to a promoter capable of directing expression of the relevant sequence. The term "operably linked" refers to a juxtaposition wherein the promoter and the coding/marker sequence are in a relationship permitting the 15 coding/marker sequence to be expressed under the control of the promoter. Thus, there may be elements such as 5' non-coding sequence between the promoter and coding/marker sequence. Such sequences can be included in the construct if they enhance or do not impair the correct control of the coding/marker sequence by the promoter.

20 The vector may be a DNA or RNA vector, preferably a DNA vector. Typically, the vector is a plasmid. Each of the sequences (i) to (iii) will typically be associated with other elements that control their expression. In relation to each sequence, the following elements are generally present, usually in a 5' to 3' arrangement: a promoter for directing expression of the sequence and optionally a 25 regulator of the promoter, a translational start codon, the coding/marker sequence, a polyadenylation signal and a transcriptional terminator.

Further, the vector typically comprises one or more origins of replication, for example a bacterial origin of replication, such as the pBR322 origin, that allows replication in bacterial cells. Alternatively or additionally, one or more eukaryotic 30 origins of replication may be included in the vector so that replication is possible in, for example yeast cells and/or mammalian cells.

-21-

The vector may also comprise one or more introns or other non-coding sequences 3' or 5' to the coding sequence or to one or more of the marker sequences. Such non-coding sequences may be derived from any organism, or may be synthetic in nature. Thus, they may have any sequence. Such sequences may be included if 5 they enhance or do not impair correct expression of the coding sequence or marker sequences.

The transfected cells are typically cultured in the presence of a heavy metal ion selected from Cd²⁺, Cu²⁺ and Zn²⁺, particularly in an amount which is not toxic to the cells. That can lead to higher expression of the desired interferon. The 10 concentration of the heavy metal ion in the culture medium is typically from 100 to 200 µM. Cells may therefore be cultured in the presence of from 100 to 200 µM of a heavy metal ion selected from Cd²⁺, Cu²⁺ and Zn²⁺, for example from 130 to 170 µM of the heavy metal ion. A useful concentration is about 150 µM, particularly when the heavy metal ion is Zn²⁺.

15 The interferon that is produced may be recovered by any suitable means and the method of recovery may vary depending on, for example, the type of cells employed and the culture conditions that have been used. Desirably, the interferon produced will be purified after recovery. Substantially pure interferon can thus be obtained.

20 The human β-interferon provided by WO 96/30531 has a high degree of sialylation. Like natural human β-interferon produced by primary diploid human fibroblasts, it is well glycosylated. However, it has a higher bioavailability than the natural β-interferon or recombinant β-interferon produced in *E.coli* (betaseron).

The higher bioavailability of the β-interferon can be characterised. When 1.5 25 x 10⁶ IU of the interferon is injected subcutaneously into the back of a rabbit of about 2 kg: (a) ≥ 128 IU/ml of the interferon is detectable in the serum of the rabbit after 1 hour, and/or (b) ≥ 64 IU/ml of the interferon is detectable in the serum of the rabbit after 5 hours.

The maximum level of interferon is typically observed after 1 hour. 30 According to (a), therefore, 128 to 256 IU/ml such as 140 to 190 IU/ml of the interferon may be detectable in the rabbit serum after 1 hour. After 5 hours according

-22-

to (b), ≥ 70 IU/ml such as ≥ 80 IU/ml of the interferon may be detectable in the rabbit serum. Typically according to (b), an amount of interferon in the range of 64 to 128 IU/ml such as 80 to 110 IU/ml can be detected.

Additionally or alternatively, the human interferon β can be characterised by 5 its specific activity. It can have a specific activity from 4.8×10^8 to 6.4×10^8 IU per mg equivalent of bovine serum albumin protein, as noted above. The specific activity may be from 5×10^8 to 6×10^8 , for example from 5.2×10^8 to 5.8×10^8 such as from 5.3×10^8 to 5.5×10^8 , IU per mg equivalent of bovine serum albumin protein.

10 The human interferon β may also be characterised by one or more of the following properties:

1. The interferon β typically has an apparent molecular weight of 26,300 as determined by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).
- 15 2. When injected as a neat intravenous bolus into a rabbit, the half life of the interferon is typically in the range of from 12 to 15 min such as about 13½ min. The bolus is injected into the rabbit ear vein and blood samples are withdrawn from the rabbit ear artery. Rabbit serum is assayed for the antiviral activity of the interferon according to the 20 modification of the method of Armstrong (1971).
3. The antiviral activity of the interferon in a human hepatoblastoma cell line (HepG2) is at least equal to and, typically, about 1.5 times the activity of natural interferon β from primary diploid human fibroblast cells. The interferon is also about 2.2 times more effective than 25 betaseron in protecting Hep2 cells against a viral challenge. Antiviral activity is again determined according to the modified method of Armstrong (1971). Actinomycin D was omitted in the antiviral determination in HepG2 cells.

The oligosaccharides associated with the interferon β of the invention may 30 also characterise the interferon β . The interferon β carries oligosaccharides which can be characterised by one or more of the following features:

-23-

1. Neutral (no acidic substituents): 5 to 15%, preferably about 10% or lower.
Acidic : 95 to 85%, preferably about 90% or higher.
2. The total desialylated oligosaccharide pool is heterogeneous with at least six distinct structural components present in the pool.
3. Matrix-Assisted Laser Desorption Ionisation - Time of Flight (MALDI-TOF) mass spectrometry and high resolution gel permeation chromatography data are summarised as follows:

10

Mass detected	Composition	Calculated Mass	gu equivalent
1786.2	5Hex, 4HexNAc, 1 2AB, Na	1782	11.1
1929.9	5Hex, 1dHex, 4HexNAc, 1 2AB, Na	1928	12.2
2295.5	6Hex, 1dHex, 5HexNAc 1 2AB, Na	2293	14.5
2660.1	7Hex, 1dHex, 6HexNAc 1 2AB, Na	2658	17.6
3019.1	8Hex, 1dHex, 7HexNAc, 1 2AB, Na	3023	20.7

The carbohydrate moiety of the human interferon β of WO 96/30531 consists of bi-, tri- and tetra-antennary complex type N-linked oligosaccharides. These 20 oligosaccharides contain repeating lactosamine(s). About 20 to 50%, for example 20 to 30%, 30 to 40% or 35 to 50%, of the oligosaccharides are bi-antennary oligosaccharides. About 30 to 65%, for example from 40 to 60% or 50 to 60%, of the oligosaccharides are tri-antennary oligosaccharides. About 2 to 15%, for 25 example from 2 to 8%, 4 to 10% or 5 to 15%, of the oligosaccharides are tetra-

-24-

antennary oligosaccharides. Percentages are calculated by weight of total analysable oligosaccharide content.

Inhibitors of the second enzyme

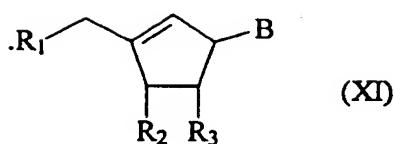
5 Compounds which are inhibitors of inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase have been reported in WO 00/50064 (the contents of which are incorporated herein by reference) to have anti-viral activity against viruses of the Flaviviridae and Rhabdoviridae families. Examples of these
10 compounds include 5-membered carbocyclic nucleosides and mycophenolic acid compounds such as those described below.

Some of these antiviral compounds have been tested as inhibitors of dihydroorotate dehydrogenase and found to be inactive. This indicates that (a) the replication of flaviviruses, rhabdoviruses and paramyxoviruses in cells involves more
15 than the inhibition of dihydroorotate dehydrogenase, and (b) dihydroorotate dehydrogenase is only one of several nucleotide synthesis enzymes necessary for viral replication.

This finding offers an opportunity for combination therapy whereby infections attributable to viruses of the Flaviviridae, Rhabdoviridae and
20 Paramyxoviridae families may be treated using a dihydroorotate dehydrogenase inhibitor and an inhibitor of a second enzyme selected from guanosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase. If desired the two inhibitors may be used in combination with an interferon. The strategy of using
25 combination therapy addresses the problem of multidrug resistance which typically arises when a single agent is used to treat a disease or disorder.

The 5-membered carbocyclic nucleoside may have the following formula (XI):

-25-



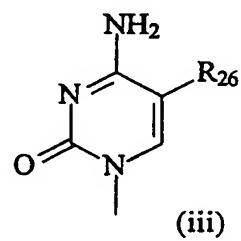
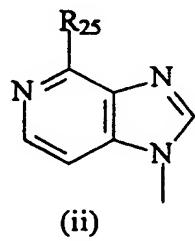
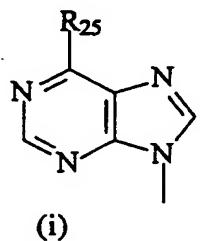
5

wherein B is selected from the group consisting of purines, pyrimidines and five- or six-membered aglycones, R₂ and R₃ are independently selected from the group

10 consisting of H, halogen, OH, O-acyl, O-aryl and O-silyl, and R₁ is as defined for R₂ and R₃ or is O-phosphate, or a pharmaceutically acceptable metabolite, metabolite derivative or salt thereof.

Preferred compounds of formula (XI) are those in which B denotes one of the following groups (i) to (viii):

15

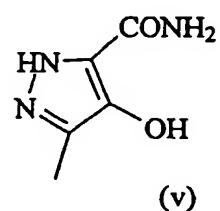
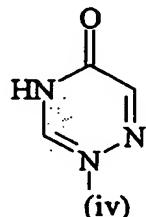


20

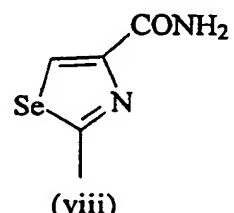
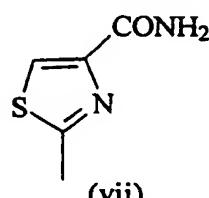
(i)

(ii)

(iii)



25



30

(vi)

-26-

wherein R_{25} is Cl or NH_2 and R_{26} is H, CH_3 , CF_3 , F, Cl, Br or I.

The 5-membered carbocyclic nucleosides of formula (XI) are known compounds and may be synthesised by published procedures or by analogy with 5 published procedures. For instance the synthesis of (-)-neplanocin A is described by Arita *et al* in J.Am. Chem. Soc. (1983), 105 (12), 4049-4055.

The synthesis of other suitable cyclopentenyl carbocyclic nucleosides is described in US-A-4975434, the contents of which are incorporated herein by reference. Specific nucleosides are listed in that patent.

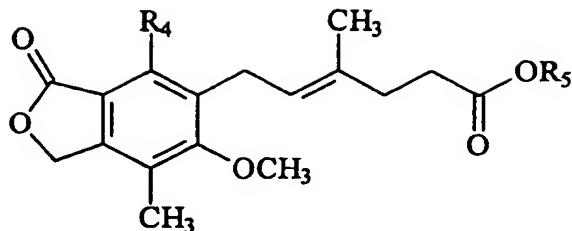
10 The substitution patterns of preferred compounds of formula (XI) are shown in the Table below. A particularly preferred compound is cyclopentenyl cytidine (CPE-C).

Substitution patterns within formula (XI)

15

R_1	R_2	R_3	Formula of Nu	Compound
OH	OH	OH	(ii) wherein R_{25} is NH_2	3-deazaneplanocin A
OH	OH	OH	(i) wherein R_{25} is NH_2	neplanocin A
20	OH	OH	(iii) wherein R_{26} is H	cyclopentenylcytidine (CPE-C)

The mycophenolic acid compounds may have the following structure (XII):



(XII)

wherein

R₄ is OR₆ or -N(R₇)R₈ in which R₆, R₇ and R₈ are independently selected from the group consisting of hydrogen and C₁-C₆ alkyl, and R₅ is selected from the group consisting of hydrogen, phenyl and C₁-C₆ alkyl unsubstituted or substituted by a five-
5 or six-membered saturated or unsaturated heterocyclic ring.

Preferred compounds of formula (XII) include those in which R₄ is hydroxy or NH₂. When one or more of R₅, R₆, R₇ and R₈ is a C₁-C₆ alkyl group, preferably it is a C₁-C₄ alkyl group such as methyl or ethyl. The five- or six-membered saturated or unsaturated heterocyclic ring generally contains one, two or three N-atoms and
10 optionally an O- and/or S-atom. Suitable such rings include pyridino, piperidino, pyrrolo, pyrrolidono and morpholino rings. A N-morpholino ring may thus be present.

Mycophenolic acid compounds suitable for use in the invention in combination with an inhibitor of dihydroorotate dehydrogenase include mycophenolic acid, mycophenolate mofetil which is the morpholinoethyl ester of mycophenolic acid, and
15 the individual mycophenolic acid derivatives described in US-A-5380879. The contents of US-A-5380879 are incorporated herein by reference.

Therapeutic uses

20 The dihydroorotate dehydrogenase inhibitors are used to treat flavivirus,

-28-

rhabdovirus and paramyxovirus infections, particularly in humans. The infection may be acute or chronic. The flavivirus may be yellow fever virus, kunjin virus, West Nile virus, dengue virus, a hepatitis virus such as hepatitis C virus, or an encephalitis virus such as St. Louis encephalitis virus, Japanese encephalitis virus, Murray valley encephalitis virus and tick-borne encephalitis virus. The rhabdovirus may be vesicular stomatis virus or rabies virus. The paramyxovirus may be respiratory syncytial virus (RSV).

A therapeutically effective amount of an inhibitor of dihydroorotate dehydrogenase is administered to a subject to be treated. The condition of the subject 10 can thus be improved. The infection may be cleared from the subject entirely.

The inhibitor can be administered in a variety of dosage forms, for example orally such as in the form of tablets, capsules, sugar- or film-coated tablets, liquid solutions or suspensions or parenterally, for example intramuscularly, intravenously or subcutaneously. It may therefore be given by injection or infusion.

15 The mode of administration and dosage regimen of the inhibitor depends on a variety of factors including the particular inhibitor concerned, the age, weight and condition of the patient and the nature of the viral infection. Typically, however, the dosage adopted for each route of administration for humans, for example adult humans, is 0.001 to 30 mg/kg, most commonly in the range of 0.01 to 5 mg/kg, body 20 weight. Such a dosage may be given, for example, daily. The dosage may be given orally or by bolus infusion, infusion over several hours and/or repeated administration.

An interferon may also be administered to the subject under treatment. The inhibitor of dihydroorotate dehydrogenase and the interferon may be given simultaneously. Alternatively they may be given up to five days from each other, for 25 example up to two days apart or up to one day apart or up to four hours apart. The relative timing of the administration of the inhibitor and the interferon may be determined by monitoring their respective serum levels. The interferon may be given before the inhibitor of dihydroorotate dehydrogenase, or *vice versa*.

The interferon may be administered in various ways such as orally, 30 intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, and subcutaneously. The particular mode of administration and dosage regimen will be

selected by the attending physician taking into account a number of factors including the age, weight and condition of the patient, the nature of the viral infection, the dihydroorotate dehydrogenase inhibitor with which it is being administered and, as required, the need to obtain a synergistic effect.

5 An inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase may be administered to the subject under treatment in addition to the inhibitor of dihydroorotate dehydrogenase, either in addition to or instead of the interferon. Suitable dosages and formulations of the

10 inhibitor of the said second enzyme are described in WO 00/50064 mentioned above.

Separate formulations of the inhibitor of dihydroorotate dehydrogenase on the one hand and of the inhibitor of the said second enzyme and/or the interferon on the other hand will generally be given to a patient. A single formulation containing each component can however be administered if the dihydroorotate dehydrogenase

15 inhibitor and the inhibitor of the said second enzyme and/or the interferon are stable in each other's presence and do not otherwise interfere with each other.

The pharmaceutical compositions that contain the interferon as an active principal will normally be formulated with an appropriate pharmaceutically acceptable carrier or diluent depending upon the particular mode of administration being used.

20 For instance, parenteral formulations are usually injectable fluids that use pharmaceutically and physiologically acceptable fluids such as physiological saline, balanced salt solutions, or the like as a vehicle which may contain physiologically acceptable amounts of organic diluents such as DMSO. Oral formulations, on the other hand, may be solids, e.g. tablets or capsules, or liquid solutions or suspensions.

25 The inhibitor of dihydroorotate dehydrogenase and the interferon, or the inhibitor of dihydroorotate dehydrogenase and the inhibitor of the said second enzyme are typically administered in such amounts that a synergistic effect is obtained. Lower doses of the two inhibitors and of interferon can be used, which results in a cost-saving and a reduction or elimination of side effects that might occur at higher doses.

30 The interferon will usually be formulated as a unit dosage form that contains from 10^4 to 10^9 , more usually 10^6 to 10^7 , IU per dose. Typically from 3×10^6 to 36×10^6 IU of

-30-

interferon is administered per day, particularly by injection such as intravenously or subcutaneously. The dosage may be administered daily, for example for up to five or up to twenty weeks.

5 The following Examples illustrate the invention.

Example 1

Test compounds were weighed and freshly dissolved in dimethyl sulfoxide (DMSO). Stock solutions of each chemical in DMSO were kept at 4° C or for longer 10 storage at minus 80° C. The stock solutions were diluted in regular medium such that the concentrations of each chemical ranged down from 100 μ M as they were serially diluted two times to nM concentrations.

Human liver (HuH7) cells, human primary fibroblasts (MRC5) and transformed monkey cells (Vero) cultured in 96 well microtitre plates were each 15 grown to near confluence in regular medium. The media was removed and each well of cells was re-incubated with 0.1 ml. of regular medium containing 1 to 2% of fetal calf serum, different concentrations of each of the chemical compounds dissolved in regular medium and an amount of each of the viruses listed in Tables 1 and 2. The controls consisted of cell controls (cells treated with chemical compounds but not with 20 virus) as well as viral controls (cells treated only with virus but not with chemicals).

Nearly all the cells (90 – 100%) in the viral control were in fact killed by the viral challenge after several days of incubation with the virus at 37° C (1 to 2 days for VSV, 5 to 6 days for yellow fever virus and 7 to 8 days for dengue fever and kunjin viruses). Cell toxicity was assessed by gross morphological changes, cell lysis and/or 25 cell cytopathic effects as monitored with the aid of a microscope 1 to 2, 5 to 6 or 7 to 8 days (to correspond to the number of days, respectively, required for VSV, yellow fever or dengue and kunjin virus to kill the cells) after the cells had been concurrently treated with a given concentration of each compound and the virus.

The results are shown in Tables 1 and 2. The antiviral activity of a compound 30 is indicated by its ED₅₀ value which is the concentration of test compound that confers a 50% protection against a 100 TCID₅₀ challenge dose of yellow fever virus, dengue

-31-

virus or kunjin virus or RSV, or against a challenge virus of vesicular stomatitis virus at an multiplicity of infection (M.O.I.) of 2.0.

-32-

Table 1: Antiviral Activities of Test Compounds

	Test compound	ED ₅₀ (μM)	Virus Challenge (TCID ₅₀)	Cell Type	Selectivity Index ^A
5	BNID	1.25	yellow fever	Vero	ND
		1.05	kunjin	Vero	
		1.25	dengue	Vero	
		1.15	yellow fever	HuH7	
		1.15	kunjin	HuH7	
		0.64	VSV	HuH7	
	Brequinar	0.02	yellow fever	Vero	2667
		0.02	kunjin	Vero	
		0.02	dengue virus	Vero	
		0.05	yellow fever	HuH7	
		0.03	yellow fever	HuH7	60*
		0.03	kunjin	HuH7	
		0.014	VSV	HuH7	2095**
10	Dichloroallyl lawsone	0.90	yellow fever	Vero	ND
		1.10	yellow fever	HuH7	
		0.78	kunjin	HuH7	
		0.49	VSV	HuH7	
	Redoxal	0.25	yellow fever	Vero	ND
		0.39	kunjin	Vero	
		0.39	dengue	Vero	
		0.63	yellow fever	HuH7	
		0.52	kunjin	HuH7	
		0.63	VSV	HuH7	
	Ribavirin	205	yellow fever	Vero	36
		309	kunjin	Vero	
		320	dengue	Vero	
		34	yellow fever	HuH7	
		60	kunjin	HuH7	70
		120	VSV	MRC5	
		250	VSV	HuH7	

A Selectivity index (SI) is estimated by the ratio of the cytotoxic dose over ED₅₀.

15 * SI @ 5 days

** SI @ 2 days

ND Not determined

-33-

Table 2: Antiviral activities of Test compounds

ED50 μ M									
	Compound	Mol Wt	VSV MRC5	VSV HuH7	YFV HuH7	KUN HuH7	YFV VERO	DEN VERO	RSV VERO
5	Brequinar	375	NA	0.08	0.03	0.04	0.02	0.04	0.02
	I3K5	343	NA	0.03	0.03	0.03	0.2	0.1	0.02
	I3K55	357	NA	NA	0.1	0.08	0.1	0.2	
	I3K46	349	NA	NA	0.1	0.1	0.1	0.5	
	I3K52	359	100	NA	0.3	0.2	1.7	NA	
10	I3K53	403		NA	0.4	0.7	0.7	12.4	
	I1K52	341	NA	NA	0.5	0.5	3.7	7.3	
	I1K46	331	NA		1	0.8	3.8	3.8	
	I1K53	385		NA	1	0.9	3.2	6.5	
	I1K55	339	168	1.9	0.5	0.2			
15	I2K5	409	NA	NA	1.7	2	0.8	0.8	
	I3K4	367	NA	NA	2.1	3.4	0.9	0.9	
	I3K36	335	NA	NA	3	3.6	1.9	3.7	
	I2K46	349	NA	NA	5.1	6.3	NA	NA	
	I2K52	425	NA	NA	11.3	9.6	NA	NA	
20	I2K55	423			6.1	5.8	11.8	11.8	
	I2K51	483	NA	NA	NA		1.3	2.6	
	I3K51	417	74		3.1	2.4	6	23.9	
	I3K44	307			10.7	15.5	32.4		
	I3K6	317			15	13.2	7.9		
25	I2K42	389				27.7			
	I2K43	363			102.9	46.5			
	I3K42	323				53			
	I2K20	353				110.7			

-34-

I3K43	297			100.9	85.1			
I1K44	289				133.9			

5

Example 2

Vero cells and HuH7 cells were grown to about 99% confluence in 96 microwells with minimum essential medium (MEM) containing 10% fetal calf serum. Growth medium was removed from the microwells and incubated with one of the

10 following:-

- (1) 100 μ l of interferon α 8 containing either 6, 3, 0.6, 0.3, 0.06, or 0.03 IU of interferon (Reference to Gb 23-902-531, NIH standard, distributed by the Natl. Inst. Allergy and Infectious Diseases, NIH, USA) per ml of
- 15 MEM containing 2.5 mg/ml human serum albumin.
- (2) 100 μ l of interferon α 2 containing either 6, 3, 0.6, 0.3, 0.06 or 0.03 IU of interferon (Reference to MRC 69/19 as well as Gb-23-902-531 standards) per ml of MEM containing 2.5 mg/ml human serum albumin.
- 20 (3) 100 μ l of interferon β containing 60, 30, 6, 3 or 0.6 IU of interferon (Reference to Gb23-902-531 standard) per ml of MEM containing 2.5 mg/ml human serum albumin.

The experiment was done in quadruplicates or duplicates. After the addition of 25 interferon to the Vero cells in the microwells, 100 μ l of a viral challenge consisting of 100 $TCLD_{50}$ yellow fever virus, kunjin virus, dengue virus or VSV was added immediately to each of the microwells. Different concentrations of test compound were included in the viral challenge as well. In the case where no test compound was used (i.e. the viral control), only 100 μ l of the viral challenge was added to the wells in 30 100 μ l of MEM containing 2.5 mg/ml human serum albumin. The test compound was Brequinar.

In the control cells treated with test compound alone, 100 μ l of MEM

-35-

containing 2.5 mg/ml human serum albumin instead of interferon was added to the cells followed by an addition of another 100 μ l of MEM/human serum albumin (2.5 mg/ml) containing test compound. The cells were examined for virus-induced cytopathic effects on days 1 to 2, 5 to 6 or 7 to 8 (to correspond to the number of days, 5 respectively, required for VSV, yellow fever or dengue and kunjin virus to kill the cells) for the antiviral activities of interferon or of the test compound. The results are shown in Table 3.

Table 3: Enhancement of Antiviral Effect of Interferon by Test Compound

Test Compound	μ M of Test Compound	Type of Human Interferon	Challenge Virus	Cell Type	Fold enhancement
5 Brequinar	0.04	α 8	yellow fever	Vero	3
	0.04	α 2	yellow fever	Vero	10
	0.04	β	yellow fever	Vero	5
	0.10	α 8	yellow fever	Vero	15
	0.10	α 2	yellow fever	Vero	20
	0.10	β	yellow fever	Vero	20
	0.10	α 2	dengue	Vero	10
	0.02	β	kunjin	HuH7	4.5
	0.02	β	yellow fever	HuH7	4.5
	0.057	β	VSV	HuH7	266
BNID	1.2	α 8	yellow fever	Vero	10
	1.2	α 2	yellow fever	Vero	5
	1.2	β	yellow fever	Vero	5
	1.2	β	kunjin	Vero	6
I2K5	0.161	α 2	yellow fever	Vero	3
I3K5	0.114	α 2	yellow fever	Vero	3
	0.114	α 8	yellow fever	Vero	3
	0.114	β	yellow fever	Vero	3
	0.160	α 2	yellow fever	Vero	10
	0.160	α 8	yellow fever	Vero	3
	0.160	β	yellow fever	Vero	3
	0.224	α 2	yellow fever	Vero	10
	0.224	α 8	yellow fever	Vero	10
	0.224	β	yellow fever	Vero	33
	0.297	α 2	Dengue	Vero	10
	0.297	β	Dengue	Vero	3
	0.417	α 2	Dengue	Vero	33
	0.417	α 8	Dengue	Vero	10
	0.417	β	Dengue	Vero	33
	0.222	α 2	Kunjin	Vero	3
	0.312	α 8	Kunjin	Vero	3

Example 3: Preparation of novel compounds of formula IIa

A mixture of the trifluoromethoxy-substituted isatin of formula (IX) defined above (0.3mmol) and potassium hydroxide (1.8mmol in 1ml of water) was heated at

-37-

125°C in ethanol (2mL) for 2 hours. A ketone of formula (X) as defined above in which R²², R³³, R⁴⁴ and R⁵⁵ are all hydrogen (0.6mmol in 1mL ethanol) was added and the mixture was refluxed further for 12 hours.

The mixture was cooled and concentrated under reduced pressure. The residue 5 was taken up in water (25mL) and extracted with diethyl ether (2 x 20mL). The aqueous layer was acidified with glacial acetic acid until precipitation occurred. The resulting precipitate of 2-(4-biphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K5) was filtered, washed thoroughly and dried in high vacuum for 2 days.

10 Characterising data for the compound are as follows:

NMR: (Acetone-d6, 400 MHz) δ: 7.42-7.54 (m, 3H), 7.76-7.79 (m, 3H), 7.88 (d, J = 8.44 Hz, 2H), 8.30 (d, J = 9.16 Hz, 1H), 8.44-8.47 (m, 2H), 8.76 (s, 1H), 8.94 (s, 1H). C₂₃H₁₄F₃NO₃ calcd. 409.1; found 410.1 (M+H)⁺.

15 By the method described above, using the appropriately substituted ketone of formula (X), the following compounds were prepared:

2-(4-biphenyl)-3-methyl-6-trifluoromethoxy-quinoline-4-carboxylic acid (I2K55);

NMR: (Acetone-d6, 400 MHz) δ: 2.44 (s, 3H), 7.49 (t, J = 7.31 Hz, 1H) (t, J = 7.59

20 Hz, 2H), 7.62 (d, J = 8.57 Hz, 1H), 7.72-7.82 (m, 6H), 7.92 (s, 1H), 8.09 (d, J = 9.16 Hz, 1H). C₂₄H₁₆F₃NO₃ calcd. 423.1; found 424.1 (M+H)⁺.

2-(4-cyclohexylphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (I2K46);

NMR: (Acetone-d6, 400 MHz) δ: 1.29-1.34 (m, 1H), 1.42-1.58 (m, 4H), 1.75-1.78 (m,

25 1H), 1.85-1.96 (m, 4H), 2.62-2.68 (m, 1H), 7.46 (d, J = 8.20 Hz, 2H), 7.78 (d, J = 9.16 Hz, 1H), 8.28-8.31 (m, 3H), 8.69 (s, 1H), 8.91 (s, 1H). C₂₃H₂₀F₃NO₃ calcd. 415.1; found 416.1 (M+H)⁺.

2-(4-benzyloxy-2-methoxy-3-methyl-phenyl)-6-trifluoromethoxy-quinoline-4-

30 carboxylic acid (I2K51);

NMR: (Acetone-d6, 400 MHz) δ: 2.29 (s, 3H), 3.58 (s, 3H), 5.27 (s, 2H), 7.07 (d, J =

-38-

8.64 Hz, 1H), 7.35 (t, J = 7.23 Hz, 1H), 7.43 (t, J = 7.48 Hz, 2H), 7.55 (d, J = 7.39 Hz, 1H), 7.77 (d, J = 8.82 Hz, 1H), 7.87 (d, J = 8.73 Hz, 2H), 7.77 (d, J = 8.82 Hz, 1H), 7.87 (d, J = 8.65 Hz, 1H), 8.27 (d, J = 9.15 Hz, 1H), 8.79 (s, 1H), 8.96 (s, 1H), $C_{26}H_{20}F_3NO_5$, calcd. 483.1; found 484.1 ($M+H$)⁺; and

5

2-(4-phenoxyphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (I2K52);
NMR: (Acetone-d6, 400 MHz) δ : 7.12-7.21 (m, 5H), 7.43 - 7.47 (m, 2H), 7.78 (d, J = 9.20 Hz, 1H), 8.27 (d, J = 9.22 Hz, 1H), 8.39 (d, J = 8.89 Hz, 2H), 8.68 (s, 1H), 8.92 (s, 1H). $C_{23}H_{14}F_3NO_4$ calcd. 425.1: found 426.1 ($M+H$)⁺.

10

15

20

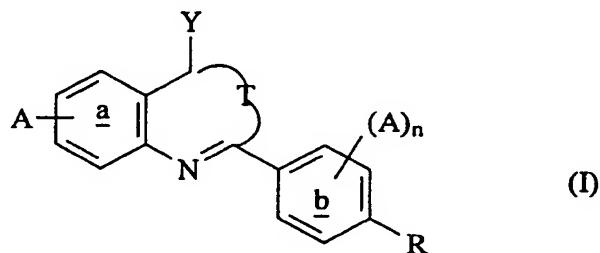
25

30

-39-

CLAIMS

1. Use of an inhibitor of dihydroorotate dehydrogenase in the manufacture of a medicament for use in the treatment of an infection attributable to a virus of the 5 Flaviridae, Rhabdoviridae or Paramyxoviridae family.
 2. Use according to claim 1, wherein the inhibitor is a compound of the formula (I):



wherein:

each A is independently selected from the group consisting of hydrogen, halogen,
10 perhaloalkoxy, amino C_1 - C_8 alkyl, NO_2 , CN , SO_2CH_3 , C_1 - C_8 alkyl, C_1 - C_8 alkoxy, C_1 - C_7 cycloalkyl, C_3 - C_7 cycloalkenyl, aryl, aryloxy, C_1 - C_6 perhaloalkyl and Y; or
two adjacent groups A on ring b form, together with the phenyl ring to which they are
attached, a naphthalene ring system;
R is cyclohexyl, phenoxy or benzoxy, or a phenyl ring which is unsubstituted or
15 substituted by a group A as defined above; or
R and an adjacent group A on ring b form, together with the phenyl ring to which they
are attached, a naphthalene or phenanthrene ring system;
Y is selected from the group consisting of $COOM$, $CONHR'$, SO_3M and hydrogen;
M is selected from the group consisting of H, Li, Na, K and 0.5 Ca;
20 R' is C_1 - C_{10} alkyl;

-40-

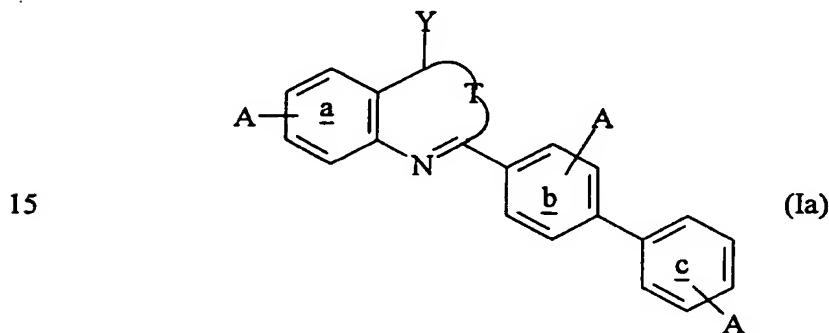
n is 1 or 2; and

T is =N- or =C(Z)- wherein either:

(i) Z is selected from the group consisting of hydrogen, NH₂, OH, C₁-C₈ alkyl, C₃-C₇ cycloalkyl, aryl and C₁-C₆ perhaloalkyl, or

5 (ii) Z is a bridging moiety selected from the group consisting of -V-W- (wherein V is CH₂ or S and W is CH₂, O, S or NH) and -(CH₂)₂-C(=Z)- wherein Z is O or H₂, the said bridging moiety being attached to the ortho position of ring b of the adjacent biphenyl group, thereby completing a ring.

3. Use according to claim 2, wherein the inhibitor is a compound of
 10 formula (Ia):



20

wherein:

each A is independently selected from the group consisting of hydrogen, halogen, amino C₁-C₈ alkyl, NO₂, CN, SO₂CH₃, C₁-C₈ alkyl, C₃-C₇ cycloalkyl, aryl, C₁-C₆

25 perhaloalkyl and Y;

Y is selected from the group consisting of COOM, CONHR', SO₃M and hydrogen;

M is selected from the group consisting of H, Li, Na, K and O.5 Ca;

R' is C₁-C₁₀ alkyl; and

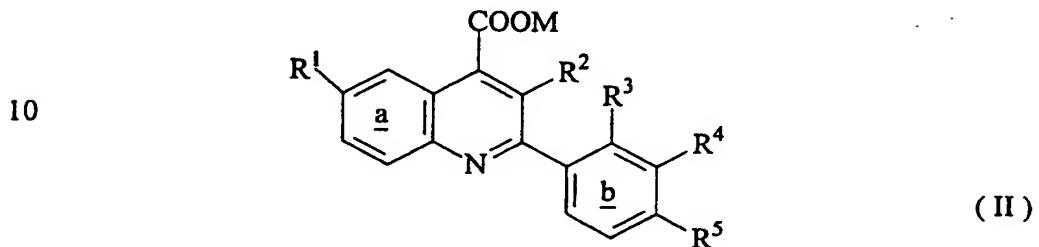
T is =N- or =C(Z)- wherein either:

30 (i) Z is selected from the group consisting of hydrogen, NH₂, OH, C₁-C₈ alkyl, C₃-C₇ cycloalkyl, aryl and C₁-C₆ perhaloalkyl, or

-41-

(ii) Z is a bridging moiety selected from the group consisting of -V-W- (wherein V is CH₂ or S and W is CH₂, O, S or NH) and -(CH₂)₂-C(=Z)- wherein Z is O or H₂, the said bridging moiety being attached to the ortho position of ring b of the adjacent biphenyl group, thereby completing a ring.

5 4. Use according to claim 2 or 3 wherein the inhibitor is a compound of the formula (II):



15

wherein

R¹ is H, a halogen or OCF₃;

R² is H or C₁-C₆ alkyl;

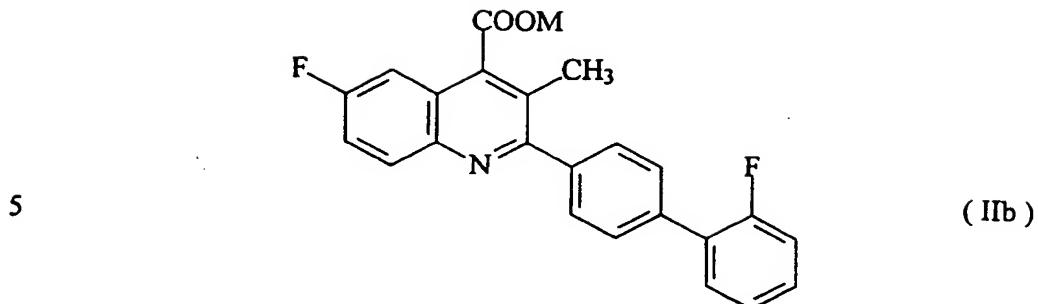
R³ is H or OR⁶ wherein R⁶ is H or C₁-C₆ alkyl;

20 R⁴ is H or C₁-C₆ alkyl; or R⁴ and R³ form, together with phenyl ring b to which they are attached, a naphthalene ring; and

R⁵ is cyclohexyl, phenyl or benzoxy, or a phenyl ring which is unsubstituted or substituted by halogen; or R⁴ and R⁵ form, together with phenyl ring b to which they are attached, a phenanthrene ring.

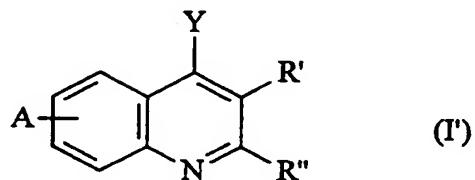
25 5. Use according to any one of claims 2 to 4 wherein the inhibitor is a compound of formula (IIb):

-42-



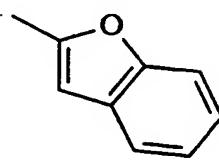
10 wherein M is H or Na.

6. Use according to claim 1 wherein the inhibitor is a compound of formula (I'):

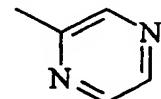


wherein A and Y are as defined above for formula (I);

15 R' is hydrogen and R'' is a thiophene ring or a group of formula (i') or (ii'):



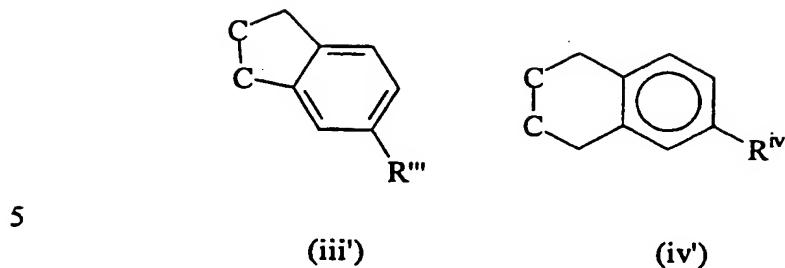
(i')



(ii')

or R' and R'' form, together with the carbon atoms (denoted "C") to which they are attached, a ring system of formula (iii') or (iv'):

-43-



5

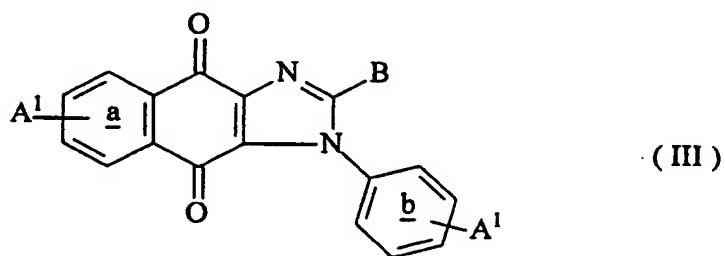
(iii')

(iv')

10 wherein R^{''} is H or halogen and R^{iv} is H or C₁ - C₆ alkoxy.

7. Use according to claim 1, wherein the inhibitor is a compound of the formula (III):

15



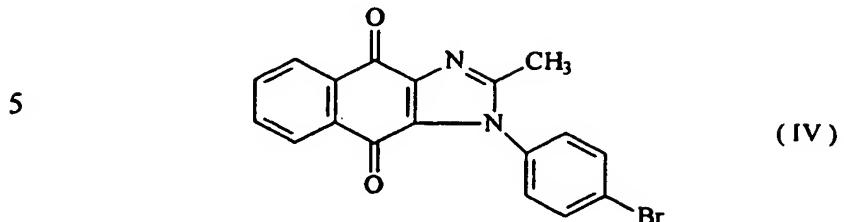
20

wherein:

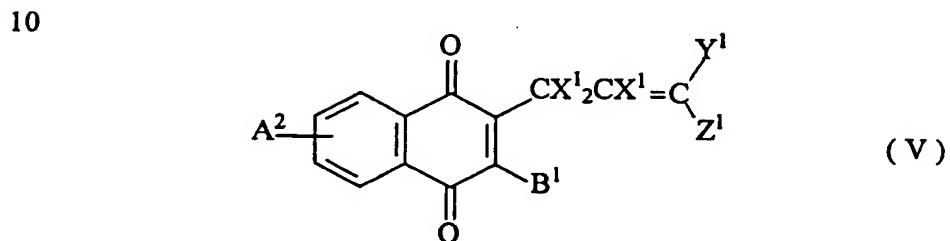
each A¹ is independently selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₈ alkoxy, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, halogen,25 unsubstituted aryl, X-substituted aryl, NO₂, CN, COOR, CONHR and NHR;X is selected from the group consisting of halogen, NO₂, C₁-C₈ alkyl, aryl, fused aryl and COOR;R is selected from the group consisting of hydrogen and C₁-C₈ alkyl; andB is selected from the group consisting of C₁-C₈ alkyl, H, CF₃ and aryl which is30 unsubstituted or substituted by halogen, C₁-C₈ alkoxy, C₁-C₈ alkyl, NO₂, aryl or fused aryl.

-44-

8. Use according to claim 7, wherein the inhibitor is a compound having the formula (IV):



9. Use according to claim 1, wherein the inhibitor is a compound having the formula (V):



15

wherein:

A^2 is selected from the group consisting of hydrogen, C_1 - C_8 alkyl, C_2 - C_8 alkenyl, C_2 - C_8 alkynyl, C_3 - C_7 cycloalkyl, halogen, unsubstituted aryl, halogen-substituted aryl, 20 fused aryl, NO_2 , CN , NHR^1 and $N(R^1)_2$;

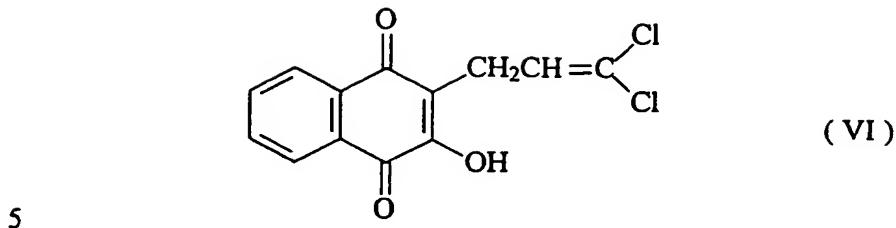
R^1 is selected from the group consisting of hydrogen, C_1 - C_8 alkyl and OH ;

X^1 is hydrogen or halogen; and

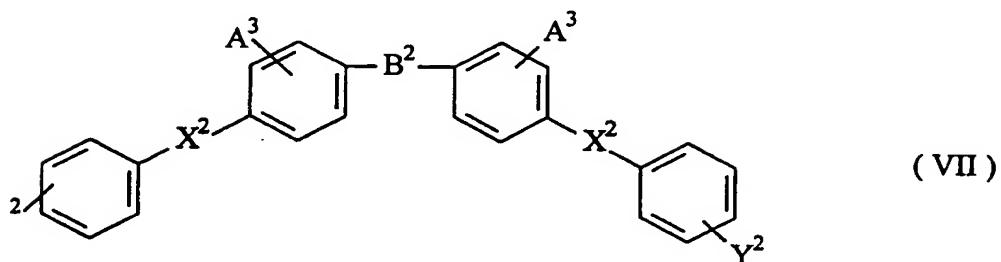
B^1 , Y^1 and Z^1 are each independently selected from hydrogen, OH , C_1 - C_8 alkyl, halogen, CN , NO_2 and CF_3 .

25 10. Use according to claim 1, wherein the inhibitor is a compound having the formula (VI):

-45-



11. Use according to claim 1, wherein the inhibitor is a compound having the formula (VII):



10 wherein:

each A³ is independently selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₁₀ alkoxy, halogen and N(R²)₂;

B² is a direct bond, -CH=CH- or -C≡C-;

X² is selected from the group consisting of O, S and NR²;

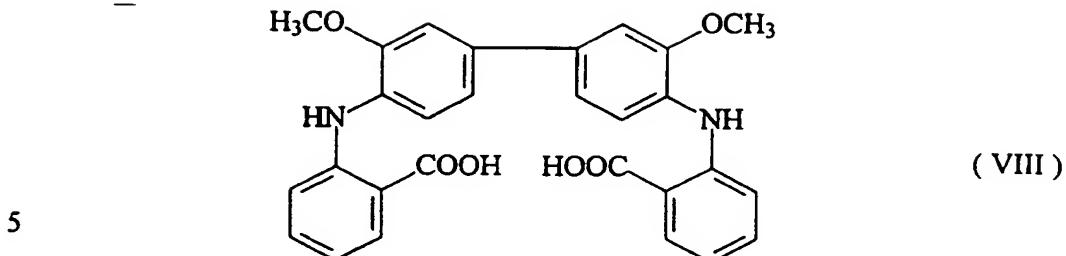
15 R² is selected from the group consisting of hydrogen, C₁-C₄ alkyl and aryl;

Y² is selected from the group consisting of COOM¹ and SO₃M¹; and

M¹ is selected from the group consisting of H, Li, Na, K and 0.5 Ca.

12. Use according to claim 11, wherein the inhibitor is a compound having the formula (VIII):

-46-



10 13. Use according to any one of the preceding claims wherein the virus is a flavivirus selected from the group consisting of hepatitis viruses, yellow fever virus, West Nile virus, kunjin virus, dengue virus, St. Louis encephalitis virus, Japanese encephalitis virus, Murray valley encephalitis virus and tick-borne encephalitis virus.

15 14. A method according to any one of claims 1 to 13, wherein the virus is a rhabdovirus selected from vesicular stomatitis virus and rabies virus, or is the paramyxovirus RSV.

15. Use according to any one of the preceding claims wherein the medicament is for administration with an interferon.

20 16. Use according to any one of the preceding claims wherein the medicament further comprises an interferon.

17. Use according to claim 15 or 16, wherein the interferon is a human interferon.

25 18. Use according to claim 17, wherein the interferon is selected from the group consisting of interferon α 2, interferon α 8 and interferon β .

19. Use according to claim 18, wherein the interferon is human interferon α 8 having a specific activity of from 0.3×10^9 to 3×10^9 IU per mg protein.

20. Use according to claim 18, wherein the interferon is human interferon β having a specific activity of from 2×10^8 to 8×10^8 per mg protein.

30 21. Use according to any one of claims 15 to 20 wherein the inhibitor and the interferon are used in respective amounts which produce a synergistic effect.

-47-

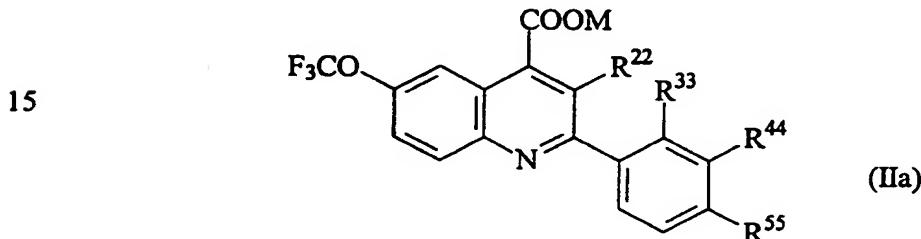
— 22. Use according to any one of the preceding claims wherein the medicament is for use with an inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase.

5 23. Use according to claim 22 wherein the medicament further comprises the inhibitor of the said second enzyme.

24. Use according to claim 22 or 23 wherein the inhibitor is mycophenolic acid, cyclopentenyl cytosine (CPE-C) or 3-deazaneplanocin A.

10 25. Use according to any one of claims 22 to 24 wherein the inhibitor of the second enzyme and the inhibitor of dihydroorotate dehydrogenase are used in respective amounts which produce a synergistic effect.

26. A compound of formula (IIa):



20 wherein

M is selected from the group consisting of H, Li, Na, K and 0.5 Ca;

R²² is H or C₁-C₆ alkyl;

R³³ is H or OR⁶ wherein R⁶ is H or C₁-C₆ alkyl;

R⁴⁴ is H or C₁-C₆ alkyl; and

25 R⁵⁵ is phenyl, cyclohexyl, phenoxy or benzoxy;

or a metabolite or prodrug precursor thereof..

27. A compound according to claim 26 which is selected from:

2-(4-biphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K5);

2-(4-biphenyl)-3-methyl-6-trifluoromethoxy-quinoline-4-carboxylic acid

30 (compound I2K55);

2-(4-cyclohexylphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound

-48-

I2K46);

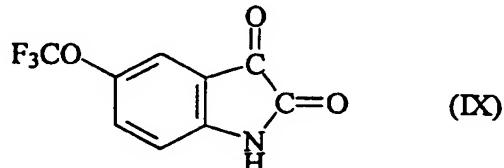
2-(4-benzyloxy-2-methoxy-3-methyl-phenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound I2K51); and

2-(4-phenoxyphenyl)-6-trifluoromethoxy-quinoline-4-carboxylic acid (compound 5 I2K52).

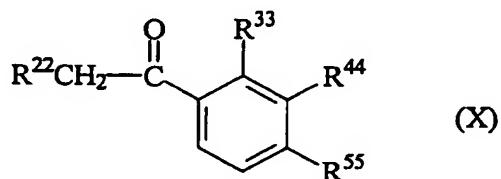
28. A process for producing a compound of formula (IIa) as claimed in claim 26, which process comprises

a) condensing a trifluoromethoxy-substituted isatin compound of the following formula (IX):

10



15 with a ketone of formula (X):

wherein R²², R³³, R⁴⁴ and R⁵⁵ are as defined in claim 26, in the presence of a base; and

(b) if desired, converting a resulting compound of formula (IIa) in which M

20 is H into a pharmaceutically acceptable salt thereof wherein M is Li, Na, K or 0.5 Ca.

29. A method of treating a host infected with a virus of the Flaviviridae, Rhabdoviridae or Paramyxoviridae family, which method comprises administering to the host an inhibitor of dihydroorotate dehydrogenase.

25 30. An anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent comprising an inhibitor of dihydroorotate dehydrogenase.

31. Products containing an inhibitor of dihydroorotate dehydrogenase and

an interferon as a combined preparation for simultaneous, separate or sequential use in treating an infection attributable to a virus of the Flaviviridae, Rhabdoviridae or Paramyxoviridae family.

32. Products containing an inhibitor of dihydroorotate dehydrogenase .
 - 5 and an inhibitor of a second enzyme selected from inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, cytidine triphosphate synthetase and S-adenosylhomocysteine hydrolase as a combined preparation for simultaneous, separate or sequential use in treating an infection attributable to a virus of the Flaviviridae, Rhabdoviridae or Paramyxoviridae family.
 - 10 33. Products according to claim 32 which additionally contain an interferon.
 34. A method for identifying an anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent, which method comprises:
 - (a) providing a test compound;
 - 15 (b) determining whether the test compound has activity as an inhibitor of dihydroorotate dehydrogenase; and
 - (c) selecting the test compound as an anti-flavivirus, anti-rhabdovirus or anti-paramyxovirus agent if it is shown to have activity in step (b).